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Mathematical modelling of methanogenic reactor start-up: Importance of volatile fatty acids degrading population

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HIGHLIGHTS

- A method was developed to evaluate microbial biomass concentration for ADM1.
- The optimized ADM1 model was used for the simulation of sludge adaptation process.
- Calibration and validation were carried out with experimental data from CSTR reactor.
- The ADM1 was able to predict the development of VFA degrading population.
- The method is suitable for modelling of reactor start-up.

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ABSTRACT

Development of balanced community of microorganisms is one of the obligatory for stable anaerobic digestion. Application of mathematical models might be helpful in development of reliable procedures during the process start-up period. Yet, the accuracy of forecast depends on the quality of input and parameters. In this study, the specific anaerobic activity (SAA) tests were applied in order to estimate microbial community structure. Obtained data was applied as input conditions for mathematical model of anaerobic digestion. The initial values of variables describing the amount of acetate and propionate utilizing microorganisms could be calculated on the basis of SAA results. The modelling based on those optimized variables could successfully reproduce the behavior of a real system during the continuous fermentation.

1. Introduction

Anaerobic digestion is a multistage process in which organic matter is decomposed to biogas, water, ammonia and other mineral compounds. Since biogas consists of carbon dioxide and methane, it may be used as fuel. Decomposition of substrate involves many groups of microorganisms. The stability of the process depends on the equilibrium between individual groups of microorganisms involved in the digestion of organic matter. The bioreactor is particularly susceptible to failure during start-up process, especially when easily biodegradable substrates are applied. The start-up procedure may be optimized with the use of mathematical models such as Anaerobic Digestion Model No. 1 (ADM1) (Batstone et al., 2002).

ADM1 is a mathematical model designed for the modelling of anaerobic digestion of sewage sludge developed by IWA task group in 2001. This model involves equations describing the biochemical processes (hydrolysis, acidogenesis, and methanogenesis) and chemical and physicochemical processes occurring during the fermentation (gas transfer, acid-base equilibrium).

This model became popular as a platform for further development of models dedicated for particular processes (Galí et al., 2009; Ntaikou et al., 2010) and was implemented in modelling software used in the development of waste treatment plants (for example SIMBA). Though particular parameters and variables have to be estimated for the modelling of individual systems. Most of the input variables (i.e. concentration of ions or concentration of substrates) for the model can be calculated on the basis of composition of culture medium. However in case of variables describing the concentration of microorganisms involved in the fermentation process the calculation of initial values is not obvious.

Traditional methods used for the determination of number of the microorganisms such as measurement of volatile suspended solids (VSS) or microscope observations do not give results which could by simply applied as model input. This problem arises from the fact that the mass of microorganisms is only a part of total

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biomass (represented by VSS) present in the bioreactor, furthermore the number of microorganisms observed under the microscope does not always reflect their metabolic activity. Thus specific anaerobic activity tests (SAA), which reflect the average activity of the sludge seem to be more appropriate for the estimation of input variables of the model. Moreover SAA are not expensive and can be prepared in laboratories with limited equipment.

Since the fermentation collapse is usually accompanied by accumulation of volatile fatty acids (VFA) (Nielsen et al., 2007) the determination of the activity of microorganisms utilizing this substrate seems to be important for the simulation of the reactor start-up. Moreover the methanogenic sludge containing higher number of syntrophic bacteria shows higher resistance during overload conditions (McMahon et al., 2004).

The goal of this work was the development of protocol based on the simple fermentation trials (SAA) for the estimation of initial value of variables describing the population of microorganisms feeding on VFA. The biogas production curves obtained from SAA on acetate, propionate and butyrate were compared with the results of the simulation based on different initial values of variables representing VFA utilizing bacteria (Xac, Xpro, Xc4). The initial values of the variables where the difference between simulation and experiment were minimal were used for the modelling of the start-up process for the laboratory bioreactor. During the fermentation experiment the development of microorganisms community was monitored with SAA and microscope observations of cells marked with fluorescently labelled oligonucleotide probes (fluorescence in situ hybridization – FISH).

2. Methods

2.1. Continuous fermentation of VFA

Fermentation was carried out in the CSTR reactor. The reactor working volume was 23 L including 20 L of fluid volume. The temperature was maintained at 37 °C by water jacket connected with the flow heater. Fresh medium was supplied with diaphragm pump (BL 1.5; Hanna Instruments). Identical pump was used for the removal of digested medium from the reactor. The temperature and pH were monitored by sensors connected to the control unit (PLC SIMATIC S7-200; Siemens). Control unit was also responsible for the management of pumps, gas flow counter and heater. The medium flow rate was set to 1 L day^{-1}, which equals to hydraulic retention time of 20 days. The gas flow was measured with ultrasound flow meter (SIMEX Sp. z o.o.). The reactor was inoculated with 10 L of anaerobic sludge from the anaerobic reactor working in the laboratory in the Biotransformation Department of the University of Wroclaw utilizing the whey, the parameters of the inoculum are presented in Table 1. The remaining volume of reactor was filled with tap water. The medium composition is presented in Table 2, for the preparation of medium tap water was used. Data from the sensors and control unit were passed to the computer and managed with Asix (Askom Sp. z o.o.) software. Samples for the determination of VFA and ammonium ions concentration were collected every 3 days. Additionally the samples taken in 10 day intervals were used for the SAA tests and FISH analysis.

Table 1
Initial sludge parameters.

Parameter	Value	Unit
Dry weight	2.47	[%]
Volatile solids	1.76	[%]
NH_4^+	1032	[mg L^{-1}]
Acetate	0.71	[mmol L^{-1}]
pH	7.05	

Table 2
Medium composition.

$NaHCO_3$	1.2	[g L^{-1}]
Urea	1.4	[g L^{-1}]
K_2HPO_4	0.2	[g L^{-1}]
Acetic acid	6–21	[cm^3 L^{-1}]
Propionic acid	4.5–15.5	[cm^3 L^{-1}]
Butyric acid	4–13.3	[cm^3 L^{-1}]

2.2. Specific anaerobic activity batch tests

The specific anaerobic activity tests were performed in 100 cm^3 serum bottles sealed with the gray rubber stoppers secured with aluminium caps. The bottles contained 67.5 cm^3 of mineral medium prepared according to Shelton and Tiedje (1984), 7.5 cm^3 of anaerobic sludge and appropriate volume of substrate stock solutions. Individual substrates used for the test were as follows (the numbers in parenthesis present the final concentration in the medium): sodium acetate (50 mmol L^{-1}), sodium propionate (25 mmol L^{-1}) or sodium butyrate (25 mmol L^{-1}). In the control samples, the substrate was omitted. After the introduction of mineral medium and anaerobic sludge, the air was removed from the head space with nitrogen and the bottles were sealed with rubber stoppers. Before the substrate was added, the samples were preincubated in 37 °C for 3 h. After this time, the substrate stock solution was added and the pressure in the bottles was equalized with the atmospheric pressure. The samples were incubated at 37 °C and were agitated manually before taking the gas production readings. The biogas production was measured every 24 h with water displacement system (Kida et al., 2001). The fermentation was monitored until the daily biogas production from the samples was equal to the production from the control. All samples were prepared in three repetitions. The SAA of the samples was calculated as the average daily biogas production in the second day of the fermentation period per 1 g of VS.

2.3. Analytical methods

Dry weight and volatile solids were measured according to Standard Methods (Clesceri et al., 1998). Ammonium concentration was measured with potentiometric method with the use of ammonium selective electrode (model EC-NH4-03, Eutech Instruments) and pH/ion benchtop meter S220 (Mettler Toledo) according to the electrode manual. The concentration of total VFA was determined with pH-metric titration method. 5 cm^3 of the sample was diluted to 50 cm^3 with deionised water and titrated with 0.05 mmol L^{-1} NaCl solution. The pH of the solution was measured with pH/ion meter S220 (Mettler Toledo). The concentration of VFA was evaluated by the comparison of volume of titration agent added between pH = 5 and pH = 4.4 with the calibration curve.

2.4. Fluorescence in situ hybridization

The samples were fixed according to the procedure described by Ariesyady et al. with modifications (Ariesyady et al., 2007). 1 ml of the sludge was homogenized in manual glass tissue grinder (Wheaton) for 5 min. The cells were collected by centrifugation (2300*g*, 5 min) and suspended in 0.9 cm^3 PBS (NaCl 130 mmol L^{-1}, sodium phosphate buffer 10 mmol L^{-1}, pH 7.2) solution. For the fixation, 0.1 cm^3 of 40% formaldehyde was added to the cell suspension. The fixation lasted for 3 h in 4 °C. After the fixation, formaldehyde was removed in the following procedure: the cells were collected by centrifugation (2300*g*, 5 min) and suspended in 1 cm^3 of PBS solution. This procedure was repeated three times. After the last centrifugation, cells were suspended in 0.5 cm^3 of

PBS and 0.5 cm^3 of 96% ethanol. Fixed samples were stored in the −20 °C before use.

The microscopic slides were prepared as follows: not homogenized sludge fragments were removed by centrifugation (25 g, 1 min.), the suspended cells were transferred to the fresh test tubes and diluted 5 times in PBS/SDS solution (NaCl 130 mmol L^{-1}, sodium phosphate buffer 10 mmol L^{-1}, pH 7.2, SDS 0.01%). 10 mm^3 of cell suspension was spread on the surface of microscopic slide and let to dry. Air dry samples were dehydrated by washing in ethanol series (50%, 80%, 96%, 100%) for 3 min each.

The probes used in the experiment are presented in Table 3. The hybridization procedure was similar to the one described by Okabe et al. (1999). The hybridization solution contained (NaCl 0.9 mol L^{-1}, Tris/HCl 0.02 mol L^{-1}, SDS 0.01%, formamide 10–50% by volume, probe 5 μg cm^{-3}). The concentration of formamide depended on the probe used. The amount of 20 mm^3 of hybridization buffer with probe was transferred on the prepared microscopic slides. The hybridization was carried in 46 °C for 90 min. After hybridization, slides were washed in washing buffer (Tris/HCl 0.02 mol L^{-1}, SDS 0.01%, EDTA 0.005 mol L^{-1}, NaCl concentration depending on the probe) for 20 min at 48 °C. After removing the washing solution with distilled water and drying slides were covered with DAPI solution (10 mg cm^{-3} DAPI, DABCO, glycerol 50%, Tris/HCl 0.01 mol L^{-1} pH = 8.75) and covered with the cover glass. Microscope slides were analyzed with the use of AxioImager.A2 (Zeiss) microscope.

2.5. Modelling and statistical analysis

The ADM1 was implemented in the Octave 3.6.4 environment. For integration of differential equations *lsode* solver was used. The applied ADM1 version included correction of the ammonium circulation introduced by Wett et al. (2006). For the initial variable value estimation, the sum of absolute error values was applied as the cost function. Downhill simplex method (Nelder–Mead (NM) algorithm) was used to find the minimum value of cost function for the set of optimized initial conditions (Nelder and Mead, 1965). The NM algorithm was implemented in the optim1.3.0 package as a function *nmsmax*. For the optimization procedure the initial value of variables representing the amount of microorganisms utilizing butyrate (Xc4), propionate (Xpro) and acetate (Xac) were chosen. As the model output compared with the experimental data, total biogas production was chosen. The initial curve fragment (time 1–5 days) was taken, since the biogas production rate remained constant for this period. The optimization procedure was done according to the following order. For each set of tested variables tree individual simulations of SAA test were run (for acetate, butyrate and propionate). Then, the obtained simulation results were compared with the experimental biogas production curves and the sum of absolute error values was calculated for each simulation. Next, the sums of errors calculated for individual substrates were added. This value was used by the NM algorithm to calculate the subsequent set of variables to be tested. Default and optimized set of variables were used as input values in the modelling of continuous fermentation process start-up. For the statistical analysis of data obtained from SAA tests, Microsoft Excel 2007 was applied.

3. Results and discussion

3.1. The estimation of maximal OLR for default simulation parameters

To asses if the default values describing the population of microorganisms (applied in SIMBA software) represent the well adapted population, the behavior of the system with various OLR was modelled. The ADM1 model implemented in the open-source software Octave could successfully simulate the fermentation process. The modelling of start-up procedure with default values (Table 4) for the microorganism concentration revealed that the system should withstand the OLR up to 7.75 gCOD L^{-1} day^{-1}. The change in the pH during the simulation of anaerobic digestion with various OLR is presented in Fig. 1. The significant decrease in pH suggesting the process collapse can be observed in the case of simulation where OLR higher than 7.75 gCOD L^{-1} day^{-1} is applied. This value is much higher than the values suggested for the start-up process in the literature (Ghangrekar et al., 2005). Thus, the values describing the composition of microorganisms population represent a well-developed community (OLR higher than 5 gCOD L^{-1} day^{-1} are usually applied in well adapted digestion systems). These values are thus inappropriate for the modelling of start-up procedure.

3.2. The optimization of model input parameters

To estimate the concentration of microorganisms from specific groups in the bioreactor, the biogas production curves from SAA trials were compared with the simulation results based on the default microorganism population parameters. For the simulation only, the gas production from first 5 days was taken. At this period, the biogas production rate is not influenced by decreasing concentration of substrate, and maximal reaction rate may be estimated (Donoso-Bravo et al., 2011). Obtained specific anaerobic activity for acetate and butyrate (0.002 mol gVS^{-1} day^{-1}) were about 60% lower than values obtained by other authors for adapted sludge samples (Fang et al., 1995). The SAA values obtained for propionate (0.0005 mol gVS^{-1} day^{-1}) were about one order of magnitude lower than the values presented in literature (Fang et al., 1995). During the anaerobic digestion with tested sludge, the oxidation of butyric acid was unlikely to be the rate-limiting step since the biogas production rate from acetate and butyrate were very similar. Thus, the total biogas production should be replaced with other parameter to estimate the number of butyrate consumers. The alternative solution may be the monitoring of butyrate uptake. This technique was applied before for the estimation of VFA uptake constants (Vavilin and Lokshina, 1996).

The gas production rate from simulation based on the default values was much higher than the one obtained from the SAA experiment for all tested substrates (Fig. 2). To find the set of initial input values describing the microorganism community, which will

Table 3
Oligonucleotide probes used in the hybridization experiments.

Name	Sequence	Specificity	Formamide concentration [%]
Eub338	GCTGCCTCCCGTAGGAGT	*Bacteria*	20
Eub338 II	GCAGCCACCCGTAGGTGT	*Bacteria*	20
Eub338 III	GCTGCCACCCGTAGGTGT	*Bacteria*	20
Smi354	CGCAATATTCCTCACTGC	*Syntrophus*	10
SynM700	ACTGGTRTTCCTCCTGATTTCTA	*Syntrophomonas*	30
Arc915	GTGCTCCCCCGCCAATTCCT	*Archae*	35
MX825	TCGCACCGTGGCCGACACCTAGC	*Methanosaeta*	50

Table 4
Initial values of dynamic variables used in the modelling of anaerobic digestion.

Dynamic variables	Symbol	Default value	Optimized value	Unit
Butyrate utilizing bacteria	Xc4	0.283	0.403	[kgCOD m^{-3}]
Propionate utilizing bacteria	Xpro	0.136	0.062	[kgCOD m^{-3}]
Acetate utilizing archaea	Xac	0.900	0.183	[kgCOD m^{-3}]

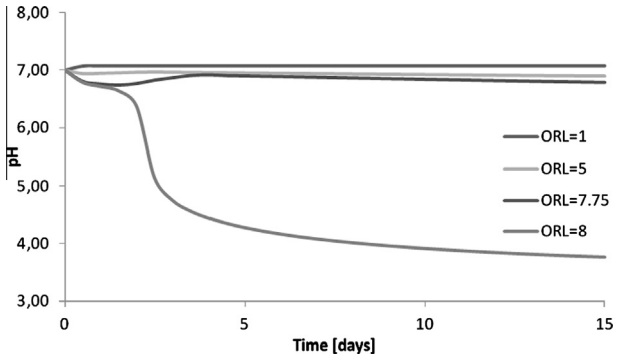

Fig. 1. The changes in pH in simulations of anaerobic digestion with various organic loading rates for default parameters of ADM1.

reproduce biogas production curves from SAA with appropriate accuracy the Nelder–Mead algorithm was applied.

The following initial dynamic state variables were optimized: Xc4, Xpro, Xac. The values obtained after the optimization process are presented in Table 4. The biogas production curves based on the optimized data reproduce the experimental results with good accuracy. The simulation deviation is lower than the variability of experimental results (Fig. 2).

The concentration of acetoclastic archaea was much lower than the one assumed in the model. The concentration of propionate utilizing bacteria was about 50% lower than the value assumed for the default model parameters. The optimized concentration of butyrate oxidizing bacteria was about 40% higher than the one assumed in the initial form of the model. The obtained value may be incorrect since the SAA test showed that oxidation of butyrate was unlikely to be rate-limiting reaction in anaerobic digestion. Thus, the monitoring of biogas production does not reflect the butyrate uptake rate (and the size of butyrate consumers' population). The estimation of maximal OLR for the system with optimized concentration of microorganisms showed that the digestion of VFA mixture collapses when OLR equal to 2.5 gCOD L^{-1} day^{-1} is applied. This value is much closer to values suggested in the literature for the start-up of anaerobic digestion (Ince et al., 1995). Since the optimized model is more susceptible for the changes in the OLR it is more appropriate for the modelling of start-up process.

3.3. Continuous fermentation experiment

To verify the accuracy of the optimized values, the continuous fermentation experiment was performed. The results of the experiment were compared with the data obtained during the simulation based on default and optimized initial dynamic state variables.

During the continuous fermentation, increasing dosage of VFA mixture was supplied to the reactor. The biogas production rate gradually increased from 0.55 mmol day^{-1} in the initial period up to 2.3 mmol day^{-1} at the end of the experiment. The gas production rate well reflects the substrate dose rate (Fig. 3A). The constant ratio between substrate dosage and biogas production indicates the stability of substrate conversion efficiency. The biogas production rate obtained from the simulation was lower than the experimental one, but showed similar trends. Simulations based on default and optimized input did not show significant difference

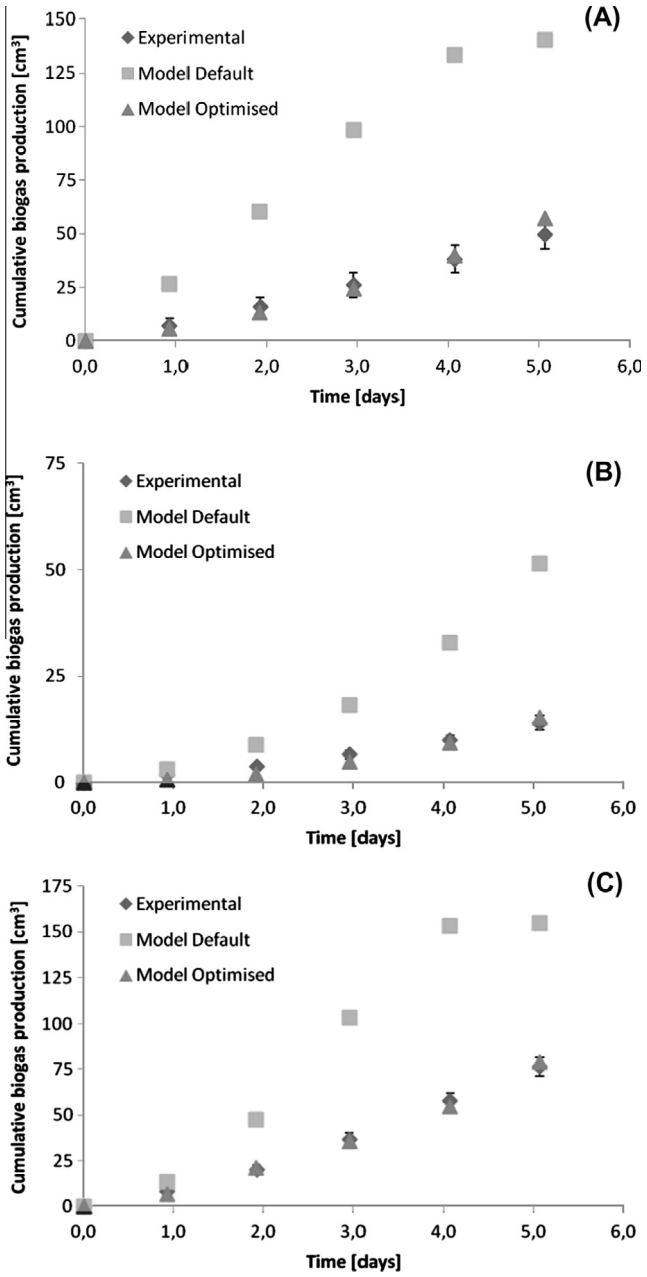

Fig. 2. Cumulative biogas production from batch fermentation tests. Tested substrates (A) acetate, (B) propionate, (C) butyrate. Error bars represent the standard deviation from the experiment.

in the biogas production rate. The lack of difference between simulation and experimental data concerning biogas production may arise from the fast adaptation of the microorganisms community to applied substrate dose rate. In such cases, the availability of substrate would become limiting for the biogas production, thus the simulations based on different initial conditions would give similar biogas production curves. The observed error between experimental and simulation data may be the result of wrong calibration of

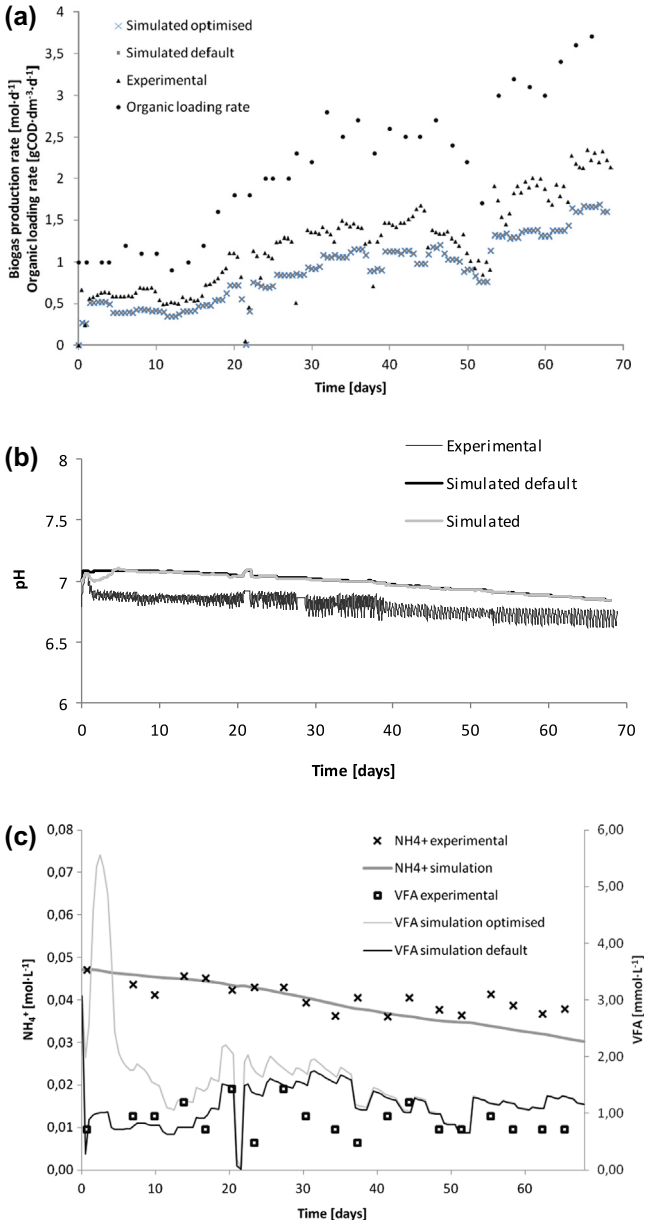

Fig. 3. Experimental and simulated data related to the continuous fermentation trial. (A) organic loading rate and biogas production rate, (B) pH, (C) ammonium ion and VFA concentration.

gas flow counter. The pH measured in the reactor was between 7.0 and 6.5 (Fig. 3B). In a short time window (1 day), the pH value showed fluctuations of 0.1 unit which are the result of semi continuous substrate dosing. For the whole experiment, the decrease in the average pH value can be observed. Similar trend was observed in the simulation, although the simulated values were about 0.1 unit higher than the one observed in the experiment. The concentration of ammonium ions showed slight decrease tendency during the experiment what was also observed in the simulation results (Fig. 3C). In the case of ammonium ion concentration, no difference between simulations based on different input values was observed. The decrease in pH may be the result of decrease in the ammonium ions concentration and lower concentration of sodium bicarbonate in the substrate in comparison to the sludge. The prolongation of the simulation of continuous fermentation showed that the concentration of cations and ammonium ions should stabilize. Thus, the fermentation should not collapse due to acidification caused by the decrease of buffering capacity.

During the fermentation process, no accumulation of volatile organic acids was observed. For the whole time of experiment, the total concentration of VFA did not exceeded 1.5 mmol L^{-1}, and usually oscillated between 0.5 and 1 mmol L^{-1}. The simulation results obtained for optimized initial parameters show sharp increase in VFA concentration in the initial period of fermentation. This phenomenon was not observed for simulation obtained with default initial parameters (Fig. 3C). The concentration of ammonium ions was in the range of 40–50 mmol L^{-1} and showed slight decrease. Simulated concentration of ammonium ions presented similar tendency.

While the production of biogas was stable and increased during the experiment, the applied OLR was sufficient for the sludge adaptation. The comparison between the simulated initial maximal OLR and the OLR applied at the end of experiment suggest that the sludge adaptation actually occurred. Moreover, the activity of microorganisms based on the SAA batch tests was not overestimated since no fermentation collapse was observed. The simulation of continuous process reproduced well the biogas production and acidity during the experiment although no significant changes in the biogas production were observed between the simulations based on default and optimized parameters. The lack of difference between simulations obtained for different initial conditions may arise from the fact that applied OLR produced conditions where the availability of substrate was limiting factor for the biogas production.

3.4. The analysis of changes in microorganisms population

To monitor the changes in the composition of microorganisms community during the continuous fermentation, the SAA batch tests were performed at the interval of 10–11 days. All tested substrates were utilized during the batch tests. Fig. 4 represents the changes in the SAA of samples taken from the continuous reactor during its operation. The production of biogas from acetate ranged between 2.3 mmol gVS^{-1} day^{-1} in the 1 day and 8 mmol gVS^{-1} - day^{-1} in 62 day of continuous fermentation. The production of biogas from the butyrate ranged from 2.6 mmol gVS^{-1} day^{-1} (day 1) to 9.8 mmol gVS^{-1} day^{-1}at 62 day of continuous fermentation. The biogas production from propionate raised from 0.5 to 2.2 mmol gVS^{-1} day^{-1}. The SAA of sludge prepared on butyrate, propionate and acetate obtained from the reactor presented statistically significant increase during continuous fermentation experiment. The observed tendency clearly shows the adaptation ability of sludge. Moreover, at the end of experiment obtained SAA values were in the same range as the ones observed in the full scale systems (Nielsen et al., 2007; Fang et al., 1995; Regueiro et al., 2012).

The biogas production from propionate was much slower in comparison to acetate and butyrate during the whole experiment. It remained about four times lower than production rates observed for other tested VFA. Similar results are observed in the literature, yet the situation where the utilization rate for acetate and propionate are similar are also observed (usually when the sludge was supplied with propionate as a sole substrate) (Fang et al., 1995).

The lower degradation rate for propionate may be the result of thermodynamics of VFA oxidation reactions (Wang et al., 1999). The Gibbs free energy for the butyrate oxidation is lower in comparison to the oxidation of propionate, thus the first reaction is favoured (Eqs. (1) and (2)).

$$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + 3H_2 + H^+$$
$$\Delta G^{\circ\prime} = 76.1 \text{ (at 25 °C)} \quad (1)$$

$$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + 2H_2 + H^+$$
$$\Delta G^{\circ\prime} = 48.1 \text{ (at 25 °C)} \quad (2)$$

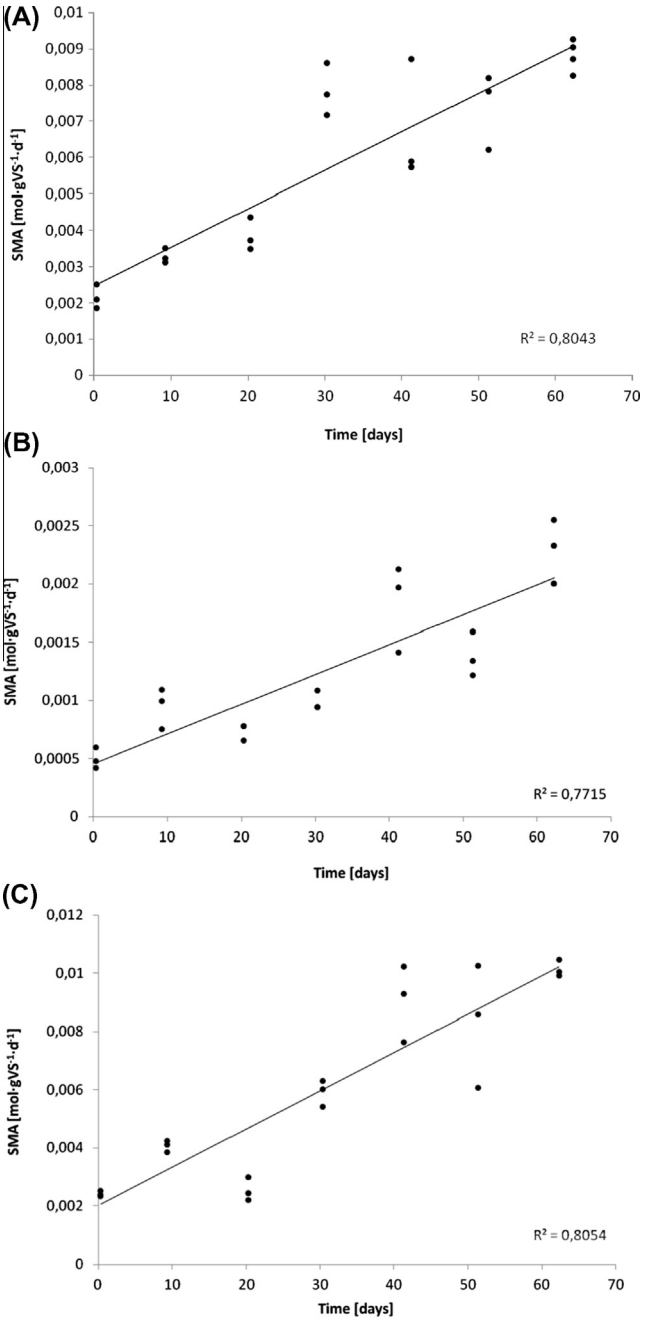

Fig. 4. The changes in specific methanogenic activity of sludge samples obtained from continuous fermentation experiment. Tested substrates: (A) sodium acetate, (B) sodium propionate, (C) sodium butyrate.

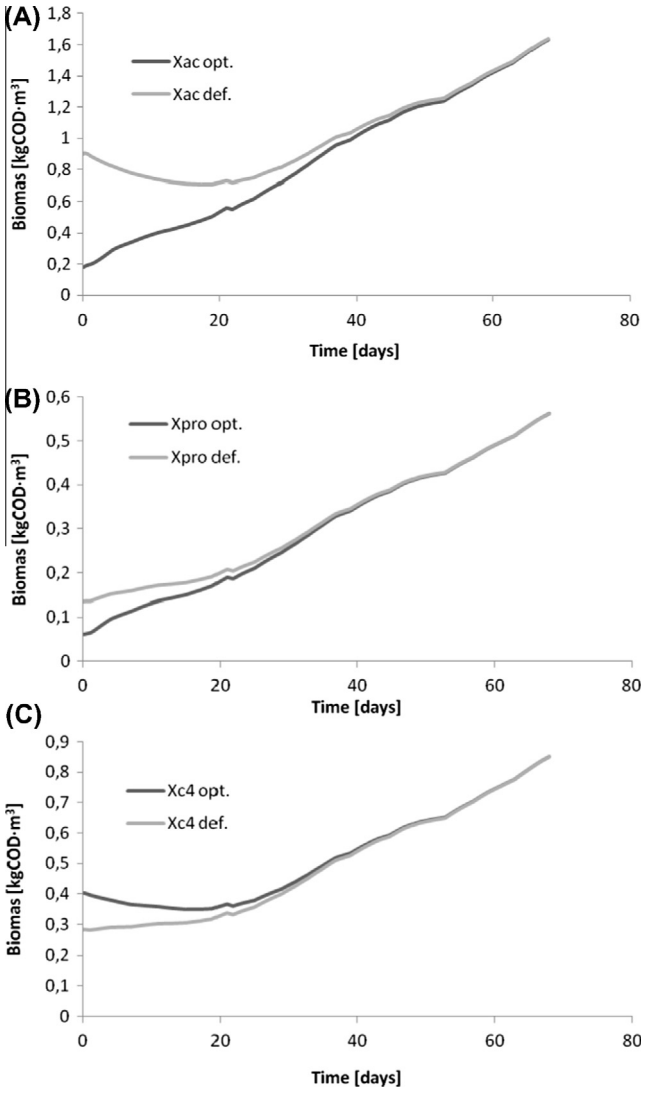

Fig. 5. The changes in concentration of microorganisms biomass. (A) Aceticlastic archaea – Xac, (B) propionate oxidizing bacteria – Xpro, (C) butyrate oxidizing bacteria – Xc4.

Another reason for propionate slow digestion may be the composition of the substrate. The reactor was fed with mixture of acetate, propionate and butyrate (COD ratio 1:1:1). It is shown that the addition of butyrate and acetate partially inhibits the oxidation of propionate (van Lier et al., 1993), thus in presented conditions, the development of bacterial community capable of propionate utilization may be slowed by the presence of acetate and propionate.

The data describing the concentration of microorganism obtained from the simulation are presented in Fig 5. In the case of propionate consumers, stable increase in the population size can be observed for both simulations (Fig. 5b). For acetate consumers, the simulation based on optimized parameters shows stable growth of population (Fig. 5a). This result is convergent with the changes in acetate SAA for samples taken during the continuous fermentation. On the other hand, for the default parameters simulation during the initial 15 days, the population is shrinking and the growth occurs after 25th day of fermentation. The opposite situation is observed in the case of butyrate consumers (Fig. 5c). This may be the result of overestimation of population size during the optimization process for butyrate oxidizing bacteria. After the 30th day, both simulations gave nearing results for all microbial communities.

3.5. Identification of microorganisms in the sludge

To evaluate which group of microorganisms is related with the changes in the activity of the anaerobic sludge, FISH technique was used. The analysis of microscope slides prepared from the samples collected for SAA tests revealed the presence of microorganisms from the *Archaea* and *Bacteria* kingdoms. The abundance of archaea remained constant for the majority of the experiment, only in the sample taken on 62nd day, the number of archaea was clearly higher. Majority of observed archaea belonged to *Methanosaeta* group. Surprisingly, Eub338 probe hybridized only with a low number of microorganisms visible in the microscope (about 10% of visible organisms). The bacteria which hybridized with probe Smi345 specific to *Syntrophus* family were observed in low num-

bers in all samples collected during the continuous fermentation. Bacteria hybridizing with the probe SynM700 specific to *Syntrophomonas* family were observed in samples taken after 30 day of continuous fermentation.

Since the sludge was capable of utilization of all tested substrates bacteria from *Syntrophomonas* family probably were not the only group of bacteria capable of butyrate oxidation. The results obtained by Ariesyady et al. (2007) indicates that butyric and propionic acids may be metabolized by bacteria belonging to β-proteobacteria.

What is somewhat surprising the archaea community consisted almost exclusively from microorganisms belonging to *Methanosaeta*. Experiments in enrichment cultures where propionate and butyrate were used as substrates revealed much diversity in the archaea population developed during cultivation (Sakai et al., 2009). On the other hand the analysis of archaea population in full scale conditions treating different types of waste showed that the *Methanosaeta* usually represent the majority (above 50%) of archaea present in bioreactors (Roest et al., 2005; Regueiro et al., 2012). Absence of archaea belonging to the *Methanosarcina* genus may be explained by low acetate concentration during the continuous fermentation experiment. Since the concentration of VFA (including acetate) was oscillating around the level of 1 mmol L^{-1}, the conditions favoured growth of archaea belonging to *Methanosaeta* (Batstone et al., 2002).

4. Conclusions

It is demonstrated that SAA in the case of acetate and propionate were sufficient to evaluate the initial state of microorganisms community utilizing these substrates. Similar estimations for butyrate oxidizing bacteria could not be achieved since their activity is not rate limiting step during the digestion of this substrate. The simulation based on the optimized parameters reproduced well the behavior of microorganisms community, which was confirmed by the analysis of SAA of samples obtained during the continuous fermentation trial. The examination of microscope slides revealed that only *Methanosaeta* related archaea could be identified with acetate consumers fraction in the ADM1.

Abbreviation index

ADM1 – Anaerobic Digestion Model No. 1,
COD – chemical oxygen demand,
CSTR – completely stirred tank reactor,
DGGE – denaturation gradient gel electrophoresis,
DAPI – 4′,6-diamidino-2-phenylindole,
DABCO – 1,4-diazabicyclo[2.2.2]octane,
FISH – fluorescence in situ hybridization,
gPCR – quantitative polymerase chain reaction,
OLR – organic loading rate,
PBS – phosphate buffered saline,
SAA – specific anaerobic activity,
VFA – volatile fatty acids,
VS – volatile solids,
Xc4 – valerate and butyrate consumers fraction,
Xpro – propionate consumers fraction,
Xac – acetate consumers fraction.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2014.09.151.

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